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ON THE MECHANISM OF GIBBERELLIN - AUXIN INTERACTION V. EFFECT OF GIBBERELLIC ACID ON THE STABILITY OF RIBONUCLEIC ACID IN BEAN HYPOCOTYL TISSUES

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Introduction

As demonstrated in earlier works, the indoleacetic acid (IAA) contrcentation in bean hypocotyls can be considerably increased by treatment with gibberellic acid (GA). In the GA-treated hypocotyl tissues, parallel with the promotion of elongation, the level of free IAA and IAA-conjugates (VARGA and BITÓ, 1968; VARGA et al., 1968) as well as the quantity of IAA-macromolecule complexes, i. e. that of IAA bound to protein and RNA (VARGA, 1968; VARGA, KÖVES and SIROKMÁN, 1968) had considerably risen.

Later on we have carried out investigations about how the resistance of RNA against ribonuclease action is influenced by the increased IAA-RNA complex formation induced by GA. According to BENDANA and GALSTON (1965) when ^{14}C-IAA is fed to excised green pea-stem segments, growth is initiated, and there is a parallel progressive labelling of the RNA extracted by cold phenol. This fraction is more resistant to degradation by ribonuclease than a similar fraction obtained from tissues not treated with ^{14}C-IAA. On the basis of these data the question arises how the RNA fraction isolated from the GA-treated bean shoots behaves towards ribonuclease. With a logical deduction it might be expected that the GA-treatment, increasing the amount of IAA-RNA complexes, result in an increased resistance of RNA against enzymatic degradation.

Materials and Method

Young seedlings of *Phaseolus vulgaris* (var. *Golden Rain*) were raised in the way described earlier (VARGA and BITÓ, 1968). The intact shoots of six-day-old green seedlings were floated, under sterile circumstances, in a culture-medium containing 0, 5 and 50 ppm GA_3, in light (8000 lux), for 24 hours. After the incubation period, the quantitative extraction of the RNA fraction was carried out with the phenol-sodium dodecylsulfate method described by DINGMAN and SPORN (1962.)

Investigation of the resistance of the RNA fraction against ribonuclease action: an aliquot of RNA fractions was incubated in 0.1 M acetate buffer (pH 5.5) with crystalline pancreatic ribonuclease (10 μg/ml), for an hour, at 35 °C. The reaction was stopped with 0.5 ml of a mixture of uranyl acetate (0.75 percent) and perchloric acid (25 percent). The reaction mixture was then filtered through a 0.45 μ filter and the optical density of the filtrate was measured at 260 nm.

The ribonuclease used was a preparation of WORTHINGTON Biochem. Co.

Results and Discussion

Our hypothesis has been supported by the results of investigations: the RNA fraction isolated from shoots treated with 5 and 50 ppm GA was hydrolyzed more slowly by crystalline pancreatic ribonuclease than the corresponding fraction from untreated tissues (Fig. 1). Between the stabilizing effect of 5 and 50 ppm GA concentrations we have found no considerable difference; the resistance of the RNA fraction of the GA-treated and control tissues against ribonuclease differed, however, significantly. This increase of resistance — with regard to the results of BENDANA and GALSTON (1965) mentioned in the introduction — can be attributed to the fact that the RNA fraction isolated from GA-treated hypocotyl tissues is much richer in IAA-complexes than the RNA fraction of control tissues. According to our data, in the hypocotyls floated on GA-solution the amount of IAA bound to macromolecules is 4 to 6 times more than that found in the untreated hypocotyls (VARGA, 1968; VARGA, KÖVES and SIROKMÁN, 1968).

FLETCHER and OSBORNE (1966) suppose that the effect of GA comes into display partly by stabilizing the nucleic acids. The present experiments concerning the stability of RNA yielded some results reminding very much of this idea.

Summary

The RNA isolated from GA-treated bean hypocotyl tissues is more resistant to degradation by ribonuclease than the similar fraction obtained from untreated

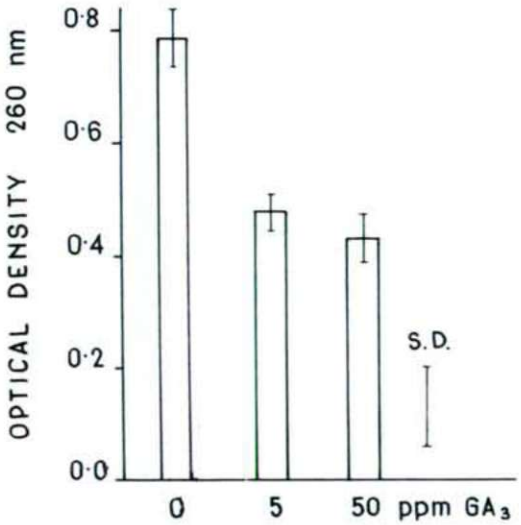

Fig. 1. Effect of GA on RNase resistance of the RNA fraction isolated from bean hypocotyls

control tissues. The increase of RNA-stability may be ascribed to the fact that in the GA-treated stem tissues the RNA fraction is much richer in IAA-complex than in the control.

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